ANALYSIS OF SODIUM TRANSPORT IN THE AMPHIBIAN OOCYTE BY EXTRACTIVE AND RADIOAUTOGRAPHIC TECHNIQUES

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ABSTRACT

The transport of Na^+ in mature *Eurycea* oocytes was studied by quantitative radioautography of $^{22}Na^+$ using techniques suitable for localization of diffusible solutes, together with conventional extractive techniques. Intracellular Na^+ consisted of three kinetic fractions: a cytoplasmic fast fraction of about 8.5 μeq/ml H_2O; a cytoplasmic slow fraction of about 58.7 μeq/ml H_2O; and a nuclear fast fraction of about 11.1 μeq/ml H_2O. A nuclear slow fraction, if it exists, does not exceed 5% of the cytoplasmic. The fast fractions represent freely diffusible Na^+ in the two compartments; the nuclear solvent space is 1.3 times the cytoplasmic. The flux of both fast fractions is determined by the permeability of the cortical membrane, with neither the nuclear membrane nor diffusion in the cytoplasm detectably slowing the flux. The cytoplasmic slow fraction is interpreted to represent Na^+ bound to nondiffusible constituents which are excluded from the nucleus; these may be yolk platelets, although the widespread observation of Na^+ binding in other cells, and the high Na^+/K^+ selectivity, argues against simple ion-binding to the yolk phosphoprotein.

INTRODUCTION

In early studies, Abelson and Duryee (1) found that in *Rana* oocytes the exchange of tracer Na^+ for endogeneous Na^+ involved two rate processes, a fast exchange with a half time of 15 min of about 12% of the cell Na^+ and a much slower exchange of the remaining cellular Na^+. These workers also developed a radioautographic method for localization of tracer Na^+ and found, qualitatively, higher grain densities over the nucleus of the oocyte than over the cytoplasm, a difference they tentatively attributed to the difference in water content of the two compartments.

Naora et al. (24) confirmed the existence of fast and slowly exchanging Na^+ fractions, as well as the higher nuclear than cytoplasmic grain density in oocytes exposed to $^{22}Na^+$ for 1 hr. Furthermore, they obtained similar radioautographs with $^{42}K^+$, $^{32}PO_4^{=}$, ^{14}C-leucine and ^{14}C-alanine, a result which might have suggested that these solutes were distributed uniformly and passively between nucleus and cytoplasm as a function of water content. However, they found by flame photometry that, on a water basis, $^{23}Na^+$ was 3.2 times and $^{39}K^+$ was 2.4 times more concentrated in the nucleus than in the cytoplasm. These results appeared to contradict the idea of a passive distribution, and suggested instead either an active transport system (12) operating across the nuclear membrane, or more likely (11) a Donnan asymmetry between nucleus and cytoplasm. Recently, however, two laboratories (5, 25), using newer techniques for the isolation of nuclei from amphibian oocytes, have reported that Na^+ in the oocyte nucleus is appreciably less concentrated than in

cytoplasm. Therefore, the question of the mechanism of control of nuclear cation concentrations as well as that of the significance and distribution of the various Na^+ exchange fractions must be reopened.

In a previous paper (15) we presented an analysis of the transmembrane and intracellular movement of a passively distributed neutral molecule, glycerol, in the amphibian oocyte, utilizing a newly developed quantitative radioautographic technique suitable for diffusible solutes. The present paper reports the results of a study of intracellular Na^+ transport using the same technique. In conjunction with microdissection and microanalysis results published elsewhere (5), this work provides a more comprehensive picture of the distribution and kinetics of oocyte sodium than has been previously possible.

MATERIALS

Oocytes

In the present study, mature oocytes of *Eurycea b. bislineata* were used. Our use of the oocytes of the plethodontid salamanders in preference to those of the conventionally used frogs, *Rana* or *Xenopus*, was dictated by the following considerations: Plethodontid oocytes are larger than frog oocytes and are less densely packed in the oviduct, which makes isolation, manipulation, and microdissection easier. The oocytes are not pigmented, and this is a substantial advantage in radioautography since pigment granules can be confused with silver grains and are dense enough in *Rana* oocyte sections to make grain density counts difficult without special treatment (15). We have found untreated plethodontid oocytes, unlike the oocytes of *Xenopus* (14), to be easily pierced by micropipettes, an important property for certain types of transport studies. Finally, plethodontid salamanders bearing mature oocytes can be obtained in the field in the eastern United States during the entire year: *Eurycea b. bislineata* from early autumn through early spring, and *Desmognathus o. ochrophaeus* in spring and summer. These are only two of the nine species of plethodontids endemic to eastern Pennsylvania (4), all of which have similar oocytes (3).

Two disadvantages of plethodontid oocytes vis a vis those of frogs should be mentioned. The relatively small number of oocytes borne by the female plethodontid make experiments requiring large numbers of oocytes more diffiult. Female *Eurycea* and *Desmognathus* only occasionally bear in excess of 35 mature oocytes. However, substantially greater numbers are found in the females of larger species as, for example, in the genera *Pseudotriton* and *Gymnophilus*. A second disadvantage is the difficulty in distinguishing, in the absence of pigment granules, the animal (nucleus bearing) hemisphere from the vegetal hemisphere in the intact oocyte. This distinction is, however, not impossible (13), and, in any case, no difficulty is experienced when examining sectioned material.

Eurycea were collected in the Perkiomen Creek drainage, Montgomery County, Pennsylvania, in November 1968 and October 1969. The salamanders were kept sexually segregated at 12°C in large preparation dishes. They were fed weekly on vestigial-winged *Drosophila*, and their dishes were cleaned and water was replaced semiweekly with spring water.

Oocytes were removed from decapitated salamanders which were pinned to a cork surface and immersed in cold Ringer's solution. Under a dissecting microscope, the ovaries were carefully slit with an iridectomy scissors to form a sheet, and the individual oocytes were isolated with a small tab of ovarian epithelium to permit handling. Adherent small oocytes were removed. Typical oocyte diameter and weight were 1.7 mm and 3.1 mg, respectively. The average per cent dry weight after drying for 2 hr at 105°C was 62.8%.

Solutions

The Ringer's solution contained 24.0 mM glucose; 92.7 mM NaCl; 2.5 mM KCl; 1.0 mM $CaCl_2$; 1.2 mM $MgCl_2$; 17.3 mM $NaHCO_3$; 2.0 mM NaH_2PO_4; 1.2 mM Na_2HPO_4. The pH was 7.2–7.3.

$^{22}Na^+$ was obtained from the New England Nuclear Corp., Boston, Mass. as carrier-free NaCl in 0.1N HCl solution containing 1.81 mCi/ml. Samples of this stock were frozen in a small vial and dried under vacuum. A portion of Ringer's solution deficient in NaCl by the appropriate amount was then added to the residual $^{22}NaCl$. The final activity was 0.25 mCi/ml.

Radioautographic Plates

Microscope slides (25 × 75 mm) coated with Kodak NTB emulsion were prepared and used as described previously (15).

METHODS

$^{22}Na^+$ Uptake

All experiments were carried out at 20.0°C. A group of 30–40 dissected oocytes were placed in a small vial with unlabelled Ringer's and allowed to come to temperature equilibrium for 1 hr. The Ringer's was drained off and replaced by $^{22}Na^+$ Ringer's; a sample was taken immediately to determine the dilution of the $^{22}Na^+$. The volume of $^{22}Na^+$-Ringer's, 1–2 ml, was large enough so that

further dilution of tracer by uptake into the oocytes was negligible. The vial was agitated continuously during the incubation. At preselected times, an oocyte was removed with a fine-tipped forceps, rinsed for 10 sec in ice-cold distilled water, blotted, weighed on a Cahn electrobalance (Cahn Instrument Co., Paramount, Calif.), and placed into 5.0 ml of doubly distilled water in a carefully cleaned polyethylene vial. The vial was tightly sealed and heated for 20 min at 100°C. This procedure extracts all of the oocyte Na^+ and K^+ into the supernatant (5). Na^+ and K^+ contents of the supernatant were determined by using a Zeiss emission flame photometer with an oxyhydrogen flame.

Additional details of the photometry procedure are provided by Century et al. (5). For determination of ^{22}Na, a 1 ml sample of the supernatant was added to 20 ml of Bray's solution, and counted in a Packard 3214 liquid scintillation spectrometer at 2°C. The standard error of the count was kept below 1%.

$^{22}Na^+$ Washout

Oocytes were loaded with $^{22}Na^+$ by incubation in $^{22}Na^+$-Ringer's as described above. Washout was initiated by removing a oocyte with fine-tipped forceps, blotting it gently and immersing it in 300 ml of Ringer's solution for 10 sec. The oocyte was then transferred through successive 5-ml portions of Ringer's with continuous agitation, for the duration of the washout. Finally the oocyte was weighed and extracted as described above. The portions of washout solution and the extraction fluid were analyzed for $^{22}Na^+$, and the time course of washout was reconstructed as described elsewhere (10).

Radioautography Technique

The radioautographic technique has been detailed elsewhere (15), and we will restrict ourselves here to describing recent improvements in the technique and modifications appropriate to its use on plethodontid oocytes with $^{22}Na^+$.

Freezing

For the plethodontid oocytes which are larger than those of *Rana*, it was found preferable to freeze the oocyte in No. 4 gelatin capsules. The capsules were ⅓ filled with O.C.T., a commercial mounting media, (Lab-Tek, Westmont, Ill.), the oocytes dropped to the surface of the O.C.T., and the remainder of the capsule was rapidly filled and the capsule was frozen. Freezing, as previously described, was carried out in dichlorodifluoromethane at −155°C. It is important in determining the final quality of the section that the oocytes not touch the capsule wall, but be entirely surrounded by O.C.T.

Sectioning and Tissue-Emulsion Contact

Sectioning was done on a Minot Custom Microtome (International Equipment Co., Needham Heights, Mass.) to which was adapted an LKB specimen chuck Model 4820B. Sectioning was done at −50°C in a Wedeen-type cryostat (Refrigeration for Science, Inc., Oceanside, N.Y.). Sections were 16 μ in thickness, and were transferred to a Teflon sheet held by an epoxy adhesive (Fluoro Plastics Inc., Philadelphia, Pa.) to a 25 × 75 mm microscope slide. Under a safelight a radioautographic plate (see Materials) was placed, emulsion downward, on the sections to form a sandwich: slide, Teflon, sections, emulsion, slide.

Exposure of the sandwich was carried out at −73°C for 117–237 hr. Exposure was terminated by separating the sandwich at room temperature.

Development and Mounting

The remaining procedure was as previously described, except that the sections were not bleached. A significant advantage of plethodontid oocytes is the absence of pigment which interferes with grain counting in *Rana* oocytes.

Quantitative Analysis of Radioautographs

Determination of grain densities was done by grain counting at 1250 × in either phase-contrast or dark-field illumination, using a Whipple-Hausser type eyepiece micrometer.

RESOLUTION: The radioautographic resolution for $^{22}Na^+$ in oocyte sections was assessed by the same method used previously (15): the edge resolution was obtained by assuming that $^{22}Na^+$ concentration is sharply discontinuous at the oocyte surface (see Results) and by determining the grain density profile through the discontinuity. A typical profile is shown in Fig. 1. The resolution, defined as in previous work as the distance over which grain density falls by 50% from its bulk value, is 45 μ. With the less conservative method used by others (2), in which resolution is defined as the distance *from the source edge* to the point at which the density is 50% of the bulk value, the resolution is 22 μ. For practical purposes, we prefer the conservative value. It is noteworthy that, despite the improved radioautographic technique, the resolution is three times poorer for $^{22}Na^+$ than for the much weaker emitter ^3H (15).

QUANTITATION: In a previous paper, some of the factors which must be controlled in relating quantitatively grain density and tracer activity in oocyte radioautographs with ^3H were described. In the present study with $^{22}Na^+$, an additional source of error associated with variable emulsion thickness is introduced, and is described below. On the other hand,

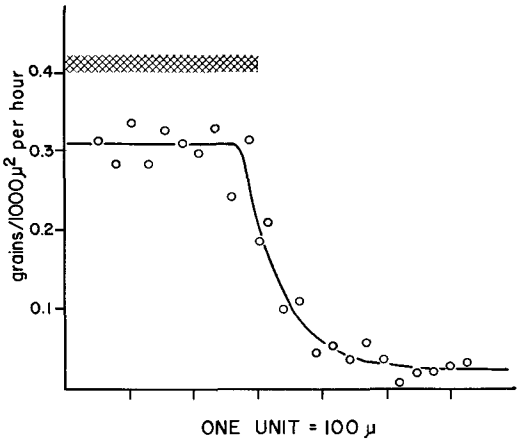

FIGURE 1 Grain density profile taken at the border of a 16 μ section of an oocyte after 2300 sec of influx in ^{22}Na-Ringer's solution and brief rinsing in Ringer's. The cross-hatched area shows the position of the overlying section.

the greater penetrating power of the $^{22}Na^+$ β can be expected to ameliorate some difficulties seen with ^3H. At a given grain density, the multi-hit error will be smaller because the grains are spread through a greater depth in the emulsion; similarly, the variation in efficiency due to local mass density variations in the section should be ameliorated. We have, nevertheless, used precautions dictated by the ^3H study in this work: specifically, we have maintained grain density below 200 grains/1000 μ^2. The question of local efficiency arises in considering the nuclear/cytoplasmic activity ratio, which, as will be seen in Results, was found from the radioautographs to be 1.3 on a water basis. A similar ratio was found for glycerol-^3H. We do not believe that this inequality is due to local efficiency variation; however, attempts to estimate local efficiency are less reliable than a direct determination of the ratio by using the new technique of Century et al. (5), which is in progress, and for present purposes we accept the raw ratio without attempted correction.

ARTIFACTS: The two types of radioautographic artifacts described previously (15) were absent in the present study. We attribute this to the various improvements in technique adopted since the original study, but forego a more detailed discussion of this matter.

A problem associated with making quantitative comparisons of grain density over sections on the same or different radioautographic plates arises when $^{22}Na^+$ or other relatively strong emitter is utilized. Leblond et al. (19) have shown that conventional dipping techniques do not yield a uniform emulsion thickness on an entire slide. When a weak β-emitter is used, this has little effect on grain density. This is not so with $^{22}Na^+$ used in the present study. For example, sections of an oocyte containing $^{22}Na^+$ were uniformly distributed on a radioautographic plate and the grain density over the sections was determined. It was found that those sections on the right side of the plate (the upper side during dipping) had a lower grain density, 104 $\pm$ 14 grains/1000 μ^2, than those on the left side of the plate, 130 $\pm$ 12 grains/1000 μ^2. The most satisfactory solution of this problem, of course, is to be found in techniques for producing uniform plates, but such techniques are not at present available (26). To circumvent the problem, we have restricted grain density counts, on sections that are to be compared to other sections, to a narrow band on each slide. This band is parallel to the short dimension of the slide, 1.0 cm wide, with its left edge 1.5 cm from the left edge of the radioautographic plate; it falls within the least steep portion of the emulsion thickness gradient.

RESULTS

Analysis by Extraction

The time course of $^{22}Na^+ - ^{23}Na^+$ exchange into oocytes is shown in Fig. 2. There is an initially rapid exchange which amounts to 10–15% of the oocyte Na^+ and which has a half time of 900–2400 sec. (These ranges relate to variation among different animals). The remainder of the Na^+ exchanges very slowly. The data in Fig. 2 indicate a half time of about 2.5 days, but the true exchange rate may be slower, since there is evidence of some deterioration at the end of a day, in that the total Na^+ level rises by 25% beginning at about 16 hr.

In order to observe the exchange kinetics with greater precision, single oocytes were loaded with $^{22}Na^+$ for different periods of time, washed out into unlabelled Ringer's, and the washout curves reconstructed. Several are shown in Fig. 3. As expected, the longer the loading period, the shallower the slope of the efflux at large times, since more of the slowly exchanging fraction has become labeled. An additional feature is seen, particularly with short loading (1500 sec), which was not resolvable in the influx curve: a small fraction exchanging with a half time of about 40–100 sec. This fraction amounts to about 1–2% of the total oocyte Na^+.

The total oocyte Na^+, then, exchanges in three

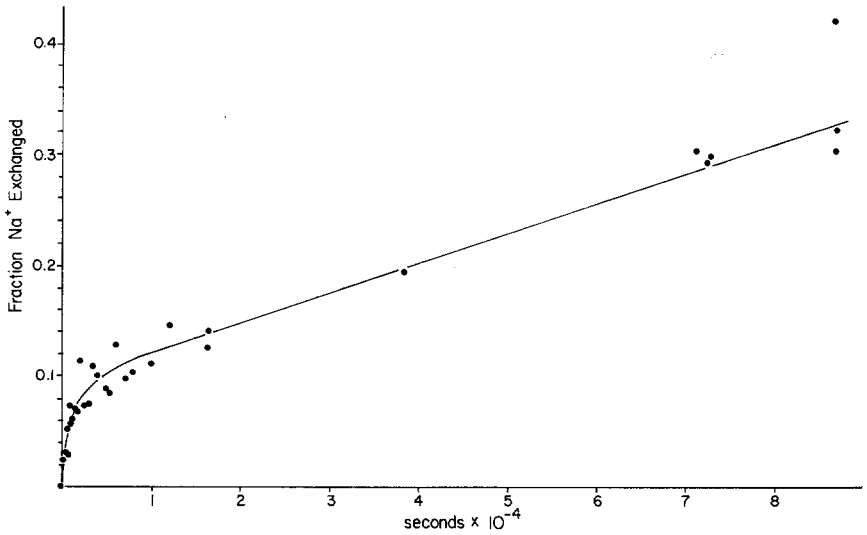

FIGURE 2 Uptake of $^{22}Na^{+}$ by oocytes of *Eurycea*, expressed as fraction of Na^{+} exchanged, as a function of influx time, t_I. Each point represents an individual oocyte on which both total Na^{+} and $^{22}Na^{+}$ were determined by extraction. Line is fitted visually.

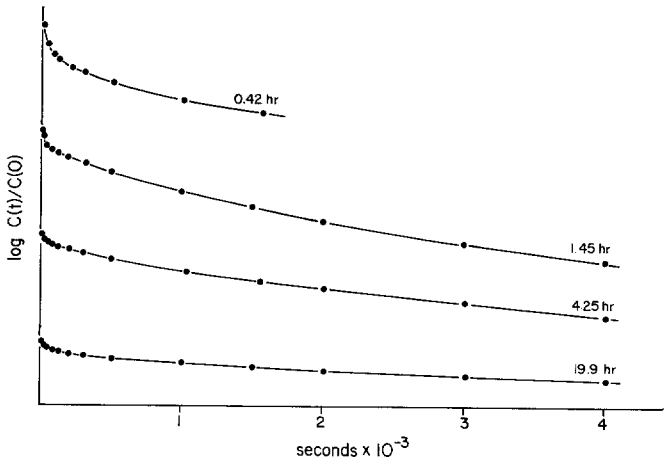

FIGURE 3 Washout of $^{22}Na^{+}$ from oocytes following loading for the indicated periods of time. Activities were determined by extraction. $C(t)/C(0)$ is the fractional activity remaining as a function of washout time. Lines are fitted visually.

kinetic fractions, which may be represented as follows:

$$1 - \frac{^{22}Na^{+}}{Na^{+}} \text{ (exchanged-in)} \qquad \text{(Equation 1)}$$

$$= 0.015\ e^{-k_1 t} + 0.125\ e^{-k_2 t} + 0.86\ e^{-k_3 t}$$

where $k_1 = 1.2 \times 10^{-2}$, $k_2 = 2.9 - 7.7 \times 10^{-4}$, and $k_3 = 3.2 \times 10^{-6}$ sec^{-1}. These rate constants were determined graphically. k_3 was determined from the limiting slope in a semilogarithmic re-plot of Fig. 2. k_2 is the final slope of washout curves in the absence of significant slow fraction (i.e., t_I = 1500 sec); k_1 is the slope of the fraction remaining when the exponential term corresponding to the fast fraction is subtracted from the same washout curves. For the oocytes of Fig. 2, the total Na^{+} content was 68.2 ± 1.3 μeq/ml H_2O; the three

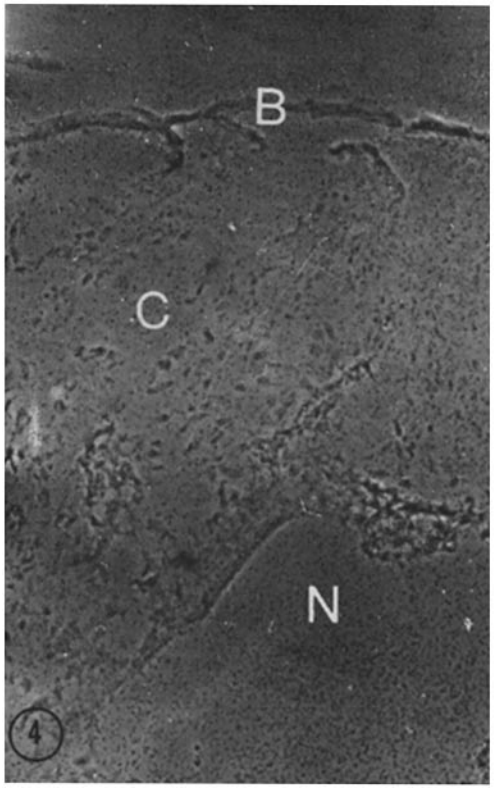

FIGURE 4 Phase-contrast view of a radioautograph of an oocyte at t_I = 1500 sec, showing oocyte boundary, *B*; cytoplasm, *C*; and nucleus, *N*. This field is that outlined by a dotted line in Fig. 5. Magnification, 155 ×.

fractions therefore constituted 1.0, 8.5, and 58.7 μeq/ml. These fractions will be referred to as the very fast, fast, and slow fractions, respectively.

Radioautography

Fig. 4 and Fig. 5 are derived from a section of an oocyte that had been loaded with $^{22}Na^+$ for 1500 sec and rinsed for 60 sec. From Equation 1, we can estimate that only 9.4 and 4.7% of the $^{22}Na^+$ in such an oocyte are in the very fast and slow fractions, respectively. The fast fraction is responsible for 85% of the activity in the cell. Fig. 4 is a phase-contrast photomicrograph of the small region indicated by a dotted rectangle on Fig. 5. Fig. 5 shows the grain density distribution over the entire section. Several observations can be made. First, the grain density does not vary significantly over the cytoplasm. Second, the grain density over the nucleus is higher than over the cytoplasm. Third, the densities are discontinuous, within the radioautographic resolution, at the oocyte surface and at the nuclear-cytoplasmic border.

The first observation, that grain density does not vary significantly over the cytoplasm, is true at all efflux times studied, from 5 to 1500 sec. This is seen in Fig. 6, which shows grain density profiles taken across the entire diameter of nucleus-free sections of oocytes washed out for various times. At 1500 sec, the fast fraction pool has decreased to only 45% of its level at 5 sec; its fraction of the total activity in the section has increased from 0.78 to 0.88 because of loss of the very fast fraction. There is no evidence of a density gradient, from the center to the circumference of the cell, of the kind that would be expected if cytoplasmic diffusion played an appreciable role in establishing the rate of fast fraction transport.

Fig. 7 shows the grain density distribution over an entire section of an oocyte loaded with $^{22}Na^+$ for 1500 sec and then rinsed for 1500 sec. The intracellular distribution of $^{22}Na^+$ is seen to have the same qualitative features as in Fig. 5; the nuclear density remains higher than cytoplasmic, and no density gradients appear in the cytoplasm.

In Fig. 8 the mean grain densities over the cytoplasm and over the nucleus are plotted as a function of washout time. The radioautographic precision does not permit kinetic curves such as in Fig. 3 to be drawn with confidence; the lines superimposed are for a 2400 sec half time. However, the data do show that the ratio of nuclear to cytoplasmic grain densities remains essentially constant throughout the washout period. The mean nuclear/cytoplasmic grain density ratio is 2.59 ± 0.08.

DISCUSSION

The Origin of the Very Fast Fraction

The fast Na^+ fraction, exchanging with a half time of 900–2400 sec, is clearly intracellular, and one may assume the same for the very large, slowly exchanging fraction. The small, very fast fraction ($t_{1/2}$ = 40–100 sec) seen in Fig. 3 cannot, however, be identified radioautographically as intracellular from the present data, and we believe that a definitive statement as to its location is beyond the present capabilities of the radioautographic technique. For example, it can easily be

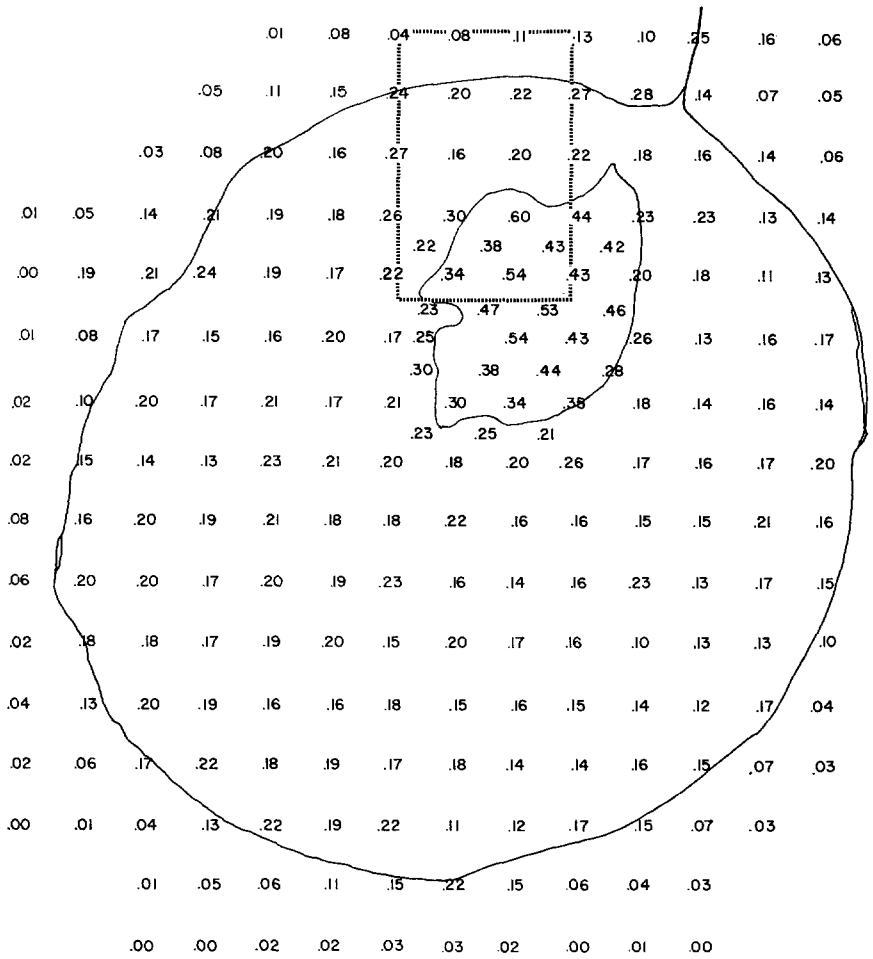

FIGURE 5 Local grain densities, in grains/1000 μ^2 per hr of radioautographic exposure, over a section of an oocyte loaded with $^{22}Na^+$ for 1500 sec and washed out for 60 sec. The field enclosed by the dotted rectangle is that of the photomicrograph in Fig. 4. Figures over the cytoplasm are 155 μ apart.

shown that a 10 μ follicle cell layer will contain enough Na^+ to account for this fraction. If the oocyte and follicle cell permeabilitues, P, are the same, the difference in size will lead to very different flux rate constants, k, by the relation

$$k = \frac{S}{V} P \qquad \text{(Equation 2)}$$

where S/V is the surface-to-volume ratio. Under these circumstances, the follicle cells need contain only as much rapidly exchangeable Na^+ per unit volume of water as the oocyte to account for the very fast fraction; radioautographically, one could see only a shift in the grain density edge by 10 μ during washout, an amount which is less than the present resolution.

On the other hand, it cannot be conclusively argued that this fraction must be extraoocytic in origin because it moves more rapidly than it could possibly diffuse out of the oocyte volume. Assuming no membrane barrier to its movement, a cytoplasmic diffusion coefficient of 8.8×10^{-6} cm^2/sec can be calculated for the very fast fraction, which is about 0.7 times the diffusion coefficient of Na^+ in water. Consequently, a model of Na^+ transport in which the cortical membrane plays no significant role, such as has been suggested for skeletal (20) and smooth muscle (17), cannot be absolutely excluded. Nevertheless, it will be seen below that the fast fraction, which constitutes 0.77–0.88 of

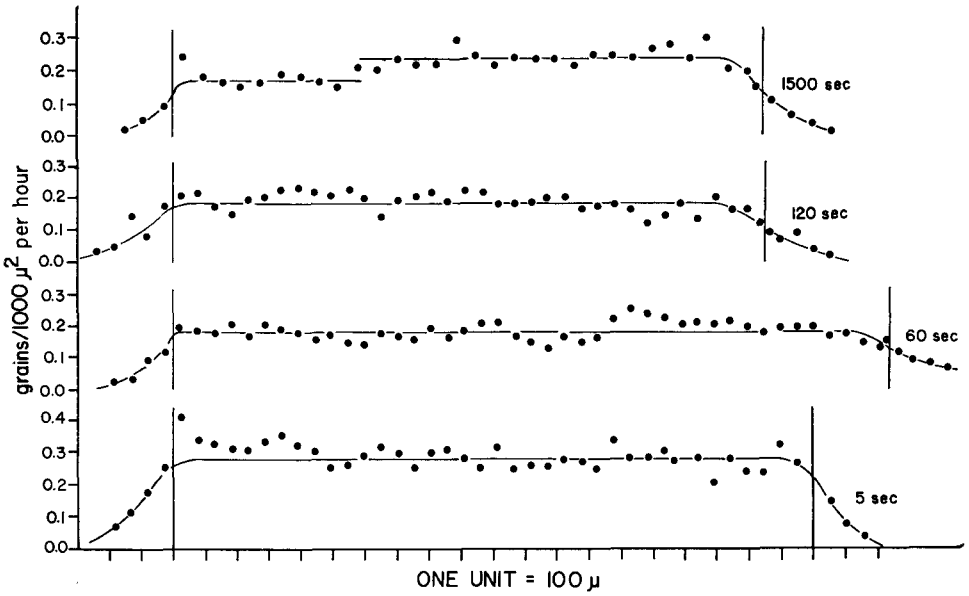

FIGURE 6 Grain density profiles through the cytoplasm of oocytes after 1500 sec of $^{22}Na^+$ uptake and various periods of washout (as indicated). The vertical lines mark the cell boundaries.

TABLE I

Sodium, Potassium, and Water Contents of Cytoplasm and Nucleus of Eurycea b. bislineata Oocytes

	Na^+ / μeq /ml H_2O	K^+ / μeq /ml H_2O	Water as per cent of total oocyte weight
Cytoplasm	83.6 ± 4.8	91.6 ± 6.7	37.2 ± 1.6
Nucleus	15.3 ± 0.6	123.4. ± 3.4	73.9 ± 2.2

Data of Century et al. (5).

the cellular $^{22}Na^+$ activity after t_I of 1500 sec, has the hallmarks of being sodium dissolved in aqueous solution, and it would seem to strain credibility to argue that the very fast fraction is also intraoocytic in origin.

Our position is to veiw the very fast fraction as having its origin outside of the oocyte.

Localization of the Slow Fraction

We have (5) measured the Na^+ and K^+ contents of the *E. bislineata* nuclei and cytoplasm by a frozen microdissection technique; these data are given in the first two columns of Table I.

The nuclear Na^+ concentration, Na_n, as determined by frozen microdissection, is on a water basis only 18% of the cytoplasmic Na_c. The radioautographic data show that the fast exchanging nuclear Na^+, per unit volume, is 2.59 times the cytoplasmic; on a water basis, using the data in the third column of Table I, the nuclear fast fraction, Na_n^f, is 1.3 times the cytoplasmic fast fraction, Na_c^f. Because the nucleus makes only a very small contribution to the total Na^+ of the oocyte, we may take the extractive data as showing that the cytoplasmic fast fraction, Na_c^f, is 10–15% of the total cytoplasmic Na^+, Na_c. We may represent these relations by the equations

$$Na_n = 0.18\ Na_c$$
$$Na_n^f = 1.3\ Na_c^f \qquad \text{(Equation 3)}$$
$$0.1\ Na_c \leqq Na_c^f \leqq 0.15\ Na_c$$

which can be solved to give

$$0.71\ Na_n \leqq Na_n^f \leqq 1.06\ Na_n \qquad \text{(Equation 4)}$$

In other words, fast exchanging Na^+ nearly

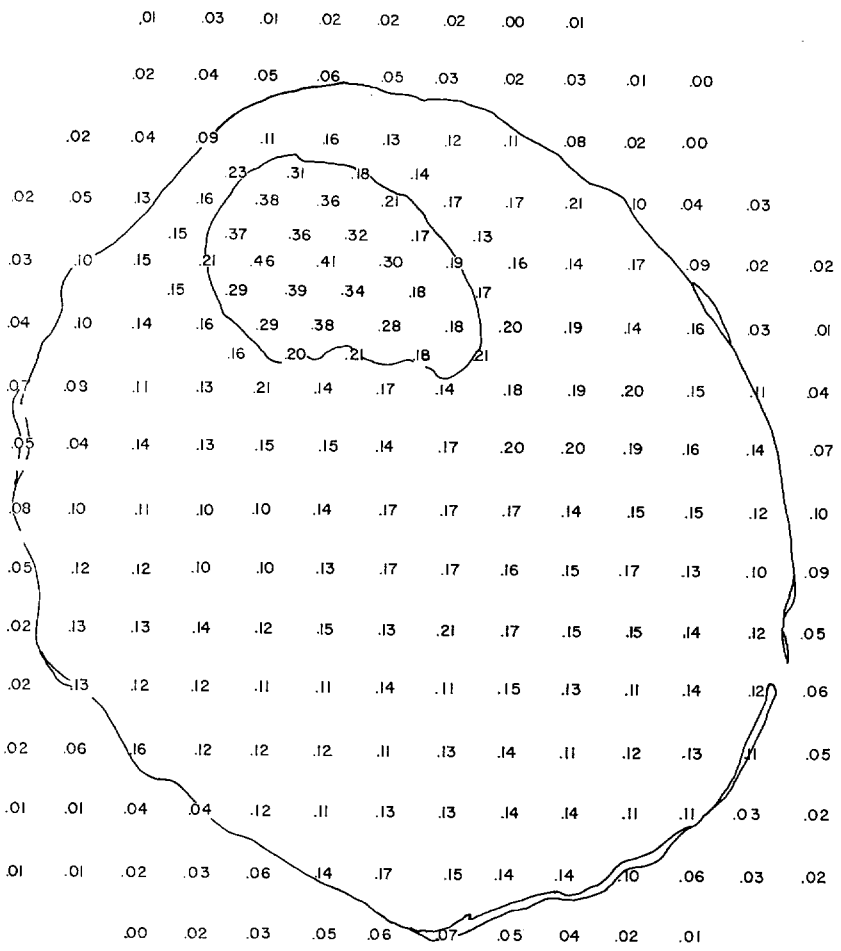

FIGURE 7 Local grain densities over a section of an oocyte loaded with $^{22}Na^+$ for 1500 sec and washed out for 1500 sec. Figures over the cytoplasm are 155 μ apart.

accounts for the entire nuclear Na^+; slowly exchanging Na^+ is either entirely absent from the nucleus or present in very small amounts. Using the lower estimate for $Na_n f$, it can be calculated from Equations 3 that the nuclear slow fraction is at most 5% of the cytoplasmic.

Cytoplasmic Na^+

We interpret the fast exchanging cytoplasmic Na^+ to be freely diffusible Na^+, and the slowly exchanging Na^+ to represent Na^+ bound to a cytoplasmic component which is excluded from the nucleus. Within the radioautographic precision, the fast fraction is uniformly distributed through the cytoplasm. No diffusional profile is seen, either after the wash-in period or during washout, contrary to the expectation based on Abelson and Duryee's estimate of 2.6×10^{-7} cm^2/sec for the diffusion coefficient of Na^+ in the cytoplasm. The absence of a diffusional profile is similar to what was observed in the case of glycerol (15); both of these materials stand in contrast to mercaptoethylamine, for which a prominent diffusional profile has been observed (16), corresponding to an apparent diffusion coefficient of 1.7×10^{-7} cm^2/sec, which seems to arise from the "chromatographic effect" (11) associated with a rapid, reversible, mixed disulfide formation between the thiol and nondiffusible cytoplasmic components.

As in the case of glycerol, the absence of a diffusional profile signifies that the rate-limiting

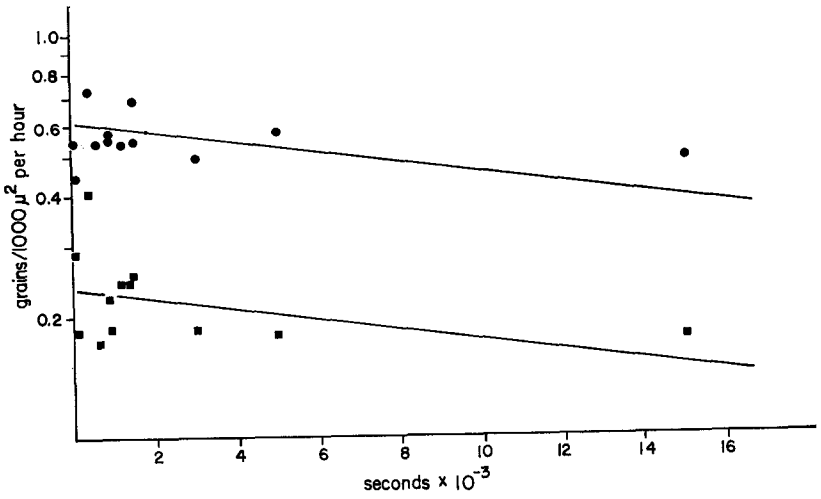

FIGURE 8 Mean grain density in nucleus (circles) and cytoplasm (squares) of oocytes as a function of t_E at 20°C. t_I was 1500 sec. Points are experimental; lines indicate 40 min half time.

step in Na^+ flux occurs at the surface. The Na^+ permeability, P_{Na}, is defined by

$$j = -P_{Na}([^{22}Na^+]_i - K_{Na}[^{22}Na^+]_o) \quad \text{(Equation 5)}$$

where j is the tracer flux in μmole/cm²-sec, $[^{22}Na^+]_i$ and $[^{22}Na^+]_o$ the $^{22}Na^+$ concentration inside and outside the cell, and K_{Na} the steady-state value of the ratio $[^{22}Na^+]_i/[^{22}Na^+]_o$. The value of P_{Na} is obtainable from the relation $k_2 = 3P_{Na}/a$, where a is the oocyte radius, and is 1.88 $\times 10^{-5}$ cm/sec. In the absence of a diffusional profile, only a minimum value of the diffusion coefficient can be estimated; this is 2.5–5 $\times 10^{-6}$ cm²/sec, which is 0.2–0.4 that of the self-diffusion coefficient of Na^+ in water (23). This rate is consistent with earlier estimates of the diffusion coefficient of water and glycerol in oocyte cytoplasm, and with a model in which this diffusion is like that in water except for a relatively weak sieving obstruction due to the solids present (11).

The rate constant for exchange of the slow fraction is about two orders of magnitude smaller than for the fast fraction. A possible origin of such a slowly exchanging fraction is the yolk platelets, which contain a crystalline array of phosphoprotein in which Na^+ counterions may be exchangeable with great difficulty. However, recent findings that Na^+ binding occurs in cells which are free of yolk suggest that a more general mechanism is involved (11). In a previous study using glycerol (15), a slowly exchanging fraction with a half time of 2.2 hr, primarily cytoplasmic, was observed, and the suggestion was offered that this fraction originated from structural elements other than the yolk platelets. The half time for Na^+ slow fraction exchange is much longer: from the data of Abelson and Duryee, about 48 hr, and from Fig. 2, about 60 hr.

The localization of the binding component will be discussed further below, in conjuncgion with an examination of the results obtained by Dick and his associates.

Nuclear Na^+

As pointed out above, the nuclear Na^+ is entirely or almost entirely exchangeable with a 900–2400 sec half time which is indistinguishable from the cytoplasmic fast fraction half time. We infer that the nuclear Na^+ is freely diffusible and that its flux is rate-limited by the cortical membrane, exchange across the nuclear membrane being rapid in comparison. This does not imply that the nuclear membrane presents no barrier to Na^+ flux. In fact, even if the nuclear membrane had the same permeability to Na^+ as the cortical membrane, the latter would appear in the present study as a much more significant barrier because of the large difference in surface-to-volume ratio of the two compartments: the flux rate constants are inversely proportional to the radii (Equation 2). The presence of a nuclear membrane Na^+ barrier is currently being investigated by a microinjection technique which bypasses the cortical membrane.

The apparent water space available to freely

diffusible Na^+ in the nucleus is 1.3 times as great as in the cytoplasm; an identical ratio was found in an earlier (15) distribution study of glycerol-^3H. The existence of cytoplasmic binding compartments provides a possible explanation for this asymmetry. Water contained within the binding compartment, in association with the bound solutes, would be expected not to serve as a solvent for freely diffusible Na^+, either because of changes in its solvent properties through interaction with bound solutes or the cytostructure or because of the existence of local transport barriers. The existence of a slowly exchanging water fraction reported in the oocyte (21) is consistent with this type of explanation.

Alternative explanations for the asymmetry of diffusible Na^+ are (*a*) a Donnan equilibrium associated with a higher nuclear fixed anion content or (*b*) active transport of Na^+ by the nuclear membrane; but we see no independent evidence for these. In this connection, the data on K^+ contents of the compartments (Table I) are informative. The nuclear/cytoplasmic ratio of *total* K^+ is 1.34, the same as the ratio of *diffusible* Na^+. This signifies either that the cytoplasmic binding is strongly selective for Na^+ over K^+ so that all of the K^+ is free or, if the binding is unselective, that the concentration of diffusible K^+ is about ninefold greater in the nucleus than the cytoplasm. The former explanation is in line with observations that the Na^+ activity coefficient in a variety of cells, including immature amphibian oocytes (9, 11), has been found to be low whereas that for K^+ is normal.

Comparison of Na^+ Transport in Immature and Mature Oocytes

Dick and coworkers (6–9, 22) have studied Na^+ in immature amphibian (*Bufo*) oocytes by a variety of techniques, including exchange, radioautography, and ion-selective microelectrodes. Immature oocytes contain a lower total Na^+ than mature. In normal Ringer's, two patterns of Na^+ exchange are observed. Exchange may occur as a single fraction with a half time of about 7 hr, or may show a slightly curved semilogarithmic plot indicative of contributions from more than one fraction. However, if Ringer's Na^+ is replaced with Li^+, the efflux kinetics decompose to a fast fraction of about 90 min half-time and a slow fraction amounting to 10–30% of the total Na^+. Radioautographs indicate that the Li^+-exchangeable Na^+ is associated with the nucleus and the yolk-free inner cytoplasm, while the Li^+-inexchangeable Na^+ is associated with the yolk-bearing peripheral cytoplasm. Microelectrode studies show the Na^+ activity coefficient to be half of that expected, while that for K^+ is normal; after washout of the Li^+-exchangeable fraction, the Na^+ activity coefficient falls to a very low value.

The characteristics of immature and mature oocytes appear to be describable by a single model, with only quantitative differences. (*a*) The immature oocyte appears to have a smaller permeability to free Na^+ but exchanges bound Na^+ more rapidly than the mature; the two processes are, as a result, only on the borderline of being kinetically distinguishable except in the presence of Li^+, which appears to increase Na^+ permeability but not to influence the exchange of bound Na^+. In the mature oocyte, the two processes are kinetically distinguishable in normal Ringer's. (*b*) Both the quantity and distribution of bound Na^+ are correlated with the yolk-bearing cytoplasm. In immature oocytes, the yolk is peripheral (18, 27), and small in amont; the bound Na^+ appears to be peripheral (6), and to constitute 10–30% of the total Na^+. In mature oocytes, the entire cytoplasm is yolky, and the bound Na^+ fraction is 85–90% of the total. (*c*) Bound Na^+ appears to be absent from the nucleus of both immature and mature oocytes; radioautographs (6) suggest that the nuclear Na^+ of the immature oocyte is comparable in concentration to the free cytoplasmic Na^+, as is true of mature oocytes.

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REFERENCES

1. Abelson, P. H., and W. R. Duryee. 1949. Radioactive sodium permeability and exchange in frog eggs. *Biol. Bull.* (*Woods Hole*). **96**:205.
2. Appleton, T. C. 1966. Resolving power, sensitivity and latent image fading of soluble-compound autoradiographs. *J. Histochem. Cytochem.* **14**:414.

3. BISHOP, S. C. 1941. The salamanders of New York. *N. Y. State Mus. Bull. 324.* 1.
4. BISHOP, S. C. 1943. Handbook of Salamanders. Comstock Publishing Co., Ithaca. i–xiv and 1–555.
5. CENTURY, T. J., I. R. FENICHEL, and S. B. HOROWITZ. 1970. The concentrations of water, sodium and potassium in the nucleus and cytoplasm of amphibian oocytes. *J. Cell Sci.* **6:** In press.
6. DICK, D. A. T., D. J. FRY, and A. W. ROGERS. 1968. Autoradiographic demonstration of sequestered fraction of Na in single oocytes of *Bufo bufo. J. Physiol. (London).* **197:** 2P.
7. DICK, D. A. T., and E. J. A. LEA. 1964. Na fluxes in single toad oocytes with special reference to the effect of external and internal Na concentration on Na efflux. *J. Physiol. (London).* **174:**55.
8. DICK, D. A. T., and E. J. A. LEA. 1967. The partition of sodium fluxes in isolated toad oocytes. *J. Physiol. (London).* **191:**289.
9. DICK, D. A. T., and S. G. A. MCLAUGHLIN. 1969. The activities and concentrations of sodium and potassium in toad oocytes. *J. Physiol. (London).* **205:**61.
10. FENICHEL, I. R., and S. B. HOROWITZ. 1963. The transport of non-electrolytes in muscle as a diffusional process in cytoplasm. *Acta Physiol. Scand.* **60** (suppl. 221)**:**1.
11. FENICHEL, I. R., and S. B. HOROWITZ. 1969. Intracellular transport. *In* Biological Membranes. R. M. Dowben, editor. Little, Brown and Co., Boston. 177.
12. GOLDSTEIN, L. 1964. Nucleocytoplasmic relationships. *In* Cytology and Cell Physiology. G. H. Bourne, editor. Academic Press Inc., New York. 559.
13. GOODALE, H. D. 1911. The early development of *Spelerpes bilineatus* (Green). *Amer. J. Anat.* **12:**173.
14. GURDON J. B. 1960. The effects of ultraviolet irradiation on uncleaved eggs of *Xenopus laevis. Quart. J. Microsc. Sci.* **101:**299.
15. HOROWITZ, S. B., and I. R. FENICHEL. 1968. Analysis of glycerol-^{3}H transport in the frog oocyte by extractive and radioautographic techniques. *J. Gen. Physiol.* **51:**703.
16. HOROWITZ, S. B., I. R. FENICHEL, B. Hoffman, G. KOLLMAN, and B. SHAPIRO. 1970. Analysis of mercaptoethylamine transport in the amphibian oocyte. *Biophys, J.* In Press.
17. JONES, A. W., and G. KARREMAN. 1969. Ion exchange properties of the canine carotid artery. *Biophys. J.* **9:**884.
18. KEMP, N. 1956. Electron microscopy of growing oocytes of *Rana pipiens. J. Biophys. Biochem. Cytol.* **2:**281.
19. LEBLOND, C. P., B. KOPRIWA, AND B. MESSIER. 1963. Radioautography as a histochemical tool. *Proc. Ist. Int. Congr. Histo. Cytochem.* 1.
20. LING, G. N. 1962. A. Physical Theory of the Living State: the Association-Induction Hypothesis. Blaisdell Publishing Co., New York. 1.
21. LING, G. N., M. M. OCHSENFELD, and G. KARREMAN. 1967. Is the cell membrane a universal rate-limiting barrier to the movement of water between the living cell and its surrounding medium? *J. Gen. Physiol.* **50:**1807.
22. MCLAUGHLIN, S. G. A. 1969. The activity and activity coefficient of Na in toad oocytes. *J. Physiol. (London)* **201:**30P.
23. MILLS, R. 1955. A remeasurement of the self-diffusion coefficients of sodium ion in aqueous sodium chloride solutions. *J. Amer. Chem. Soc.* **77:**6116.
24. NAORA, H., H. NAORA, M. IZAWA, V. G. ALLFREY, AND A. E. MIRSKY. 1962. Some observations on differences in composition between the nucleus and cytoplasm of the frog oocyte. *Proc. Nat. Acad. Sci. U.S.A.* **48:**853.
25. RIEMANN, W., C. MUIR, and H. C. MACGREGOR. 1969. Sodium and potassium in oocytes of *Triturus cristatus. J. Cell Sci.* **4:**299.
26. ROGERS, A. W. 1967. Techniques of Autoradiography. Elsevier Publishing Co., Amsterdam. i–xi and 1–335.
27. WISCHNITZER, S. 1966. The ultrastructure of the cytoplasm of the developing amphibian egg. *In* Advances in Morphogenesis. M. Abercrombie and J. Brachet, editors. Academic Press Inc., New York. 131.